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A Decision Support Tool based on microbial safety prediction for a better dimensioning of modified atmosphere packaging

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ABSTRACT

Predicting microbial safety of fresh products in Modified Atmosphere Packaging (MAP) systems implies to take into account the dynamic of O₂ and CO₂ exchanges in the system and its effect on microbial growth. In this purpose we coupled mathematical models of gas transfer (permeation through packaging and solubilisation / diffusion within food) with predictive microbiology models that take into account the effect of CO₂ and O₂ partial pressure in headspace and corresponding dissolved concentrations in the food. This mechanistic model was validated in simplified and in real conditions using dedicated challenge-tests performed on poultry meat, fresh salmon and processed cheese, inoculated with either *Listeria monocytogenes* or *Pseudomonas fluorescens*.

Once validated, this model could be used as a Decision Support Tool in order to optimize the initial packaging atmosphere (level of O₂ and CO₂) and / or the geometry (ratio headspace volume to food mass). This tool could also be used to identify the packaging gas permeability the most suitable for maintaining the targeted % of gas initially flushed in the pack within a given tolerance. This approach permits a better dimensioning of MAP of fresh produce by selecting the packaging material fitted to "just necessary" (and not by default the most barrier one). The connexion of this model with dedicated databases gathering gas permeabilities of commonly used packaging materials allows us to obtain as output a ranking of the most suitable materials. This tool would be very useful for all stakeholders of the fresh produce chain. A demonstration of this Decision Support Tool is here proposed with the pipeline between mathematical models and related databases.

Keywords: mass transfer; predictive microbiology; Decision Support Tool; Modified Atmosphere Packaging; CO₂ dissolution

INTRODUCTION

Ensuring microbial safety in active Modified Atmosphere Packaging (MAP) packed food relies on the use of a mixture of gases that limit microorganism growth. High content of CO₂ (> 20% vol/vol) are usually used for its inhibiting effect on bacterial growth combined or not with the removal of O₂ that inhibits aerobes growth (Chaix et al., 2015b; Gill and Ahvenainen, 2003; Mcmillin, 2008; Zhao et al., 1995). The maintaining during storage of its initial internal atmosphere relies on the dynamic of gas exchange in the food/packaging system. CO₂ content decreased as a function of time due to the dissolution of this gas in the food product and to its permeation through the packaging material toward external atmosphere. On the contrary, when O₂ is removed from the pack, O₂ initially dissolved in the food product is released into the headspace and tend to enter the pack via permeation from external atmosphere. Therefore, O₂ concentration in headspace tends to increase as a function of time. The rate of the both aforementioned phenomena depends on the values of diffusivity and permeabilities of gases (O₂/CO₂) within the food product and the packaging

material respectively (Chaix et al., 2014). Predicting microbial safety in MAP necessitates thus to take into account the dynamic of gas exchanges in mathematical model and its impact on predictive microbiology, which was never taken into account up to now. If existing tools of predictive microbiology (Symprevius, Combase etc.) consider in some cases CO₂ effect on microbial growth, it is always a value kept constant (e.g. initial concentration flushed in the pack) and they never consider the O₂ effect on aerobes.

In order to propose a decision support tool in the field of food packaging aiming at helping the food manufacturer to properly designed his MAP system, we have developed a semi-mechanistic model coupling O₂/CO₂ transfer equations and predictive microbiology models (Chaix et al., 2015a). This model takes into account O₂/CO₂ permeation through the packaging material, the O₂/CO₂ dissolution/diffusion into the food product and the impact of O₂/CO₂ headspace partial pressure and gradient into food on microbial growth. It was successfully validated on a simplified system (model cheese) with *Listeria monocytogenes* as targeted microorganism (Chaix et al., 2015a). The objective of the present presentation is to present a validation of this model using dedicated challenge test on poultry meat and cheese inoculated with two different microorganisms *Listeria monocytogenes* and/or *Pseudomonas fluorescens*. Once validated, this type of model is very useful to optimize the initial packaging atmosphere (level of O₂ and CO₂ to flush in the pack) and / or the geometry (ratio headspace volume to food mass) of the system. It could be used also in a reverse manner to help the user to select its packaging material by querying dedicated databases. A demonstration of this Decision Support Tool with query of related database using the @Web tool (Buche et al., 2011) is done in this paper. This integrated, reasoned approach permits to choose the "just necessary" packaging material and not by default the most barrier one which is also the most expensive and the most impacting on environment (usually multilayers that could not recycled).

MATERIALS & METHODS

Packaging materials and permeability. Two different packaging systems were used for their representativeness of real (industrial) case study. PS/EVOH/PS tray plus OPET/EVOH/PE lid film was selected for poultry meat and APET/PE tray plus PE lid film for cheese system. Oxygen O₂ and CO₂ permeabilities for each lid film or tray were determined according to the ASTM D3985 by LNE laboratory (France).

Food samples, inoculation and growth data. The poultry meat was obtained from a local manufacturers and processed cheese was produced in this study as described elsewhere (Chaix et al., 2015c). Challenge tests were performed using a method adapted from (Augustin et al., 2011) by two laboratories (Aerial and Actalia, France). Prior contamination, poultry meat was ionised 7.5 kGy using Aerial facilities to inhibit the development of annex microflora. Poultry meat was artificially contaminated on food surface with 0.5 ml of diluted subcultures of *L. monocytogenes* (ADQP105 strain isolated from smoked salmon, and provided by ADRIA Développement, Quimper, France) and *P. fluorescens* (ADRIA Développement, Quimper, France) in order to obtain an initial concentration of approximately 10² cfu/g for each microorganism. Both microorganisms were inoculated in the same pack on two separated pieces of meat. In the case of processed cheese, contamination was done in the mass (just after renneting and before gel formation) with *P. fluorescens*. Control samples without inoculation with *L. monocytogenes* and *P. fluorescens* were also included. Contaminated packed foods were stored at either 4, 8 and 15°C and enumerations of *L. monocytogenes* and *P. fluorescens* were performed during storage according to the ISO 11290-2 standard on three samples at approximately 10 different times during the lag, the exponential and the stationary phases of the growth curve. Various Modified Atmosphere Packaging systems were used for each product: 50% CO₂/50%N₂ for poultry meat and 60% CO₂/10%O₂/30% N₂ and 30% CO₂/70% N₂ for cheese. Air at 15°C was used as control and for determining optimal growth rate required for the simulation.

pH and water activity (aw) of foods were measured by the participating laboratories to characterize the variability of the physico-chemical characteristics of the studied foods. Aerobic microorganisms and mesophilic lactic acid bacteria were also enumerated at the beginning of the storage according to the ISO standards

Mathematical model. The mathematical model used here to simulate gas exchange in the packed food (including permeation through the packaging material, solubilisation and diffusion into the food) concomitantly with the microbial growth was previously described in (Chaix et al., 2015a). This model took into account temperature effect through Arrhenius equation applied to all physical mechanisms (solubility,

diffusion, permeation). The specificity of the updated model used here was that the net production/consumption of gases due to respiration was non-null and intervened in the mass balance of gases in headspace:

$$V_{HS} \frac{dC_{j,HS}}{dt} + C_{j,HS} \frac{dV_{HS}}{dt} = \varphi_{j,L} + \varphi_{j,I} + S_{j,F} \quad (1)$$

where $C_{j,HS}$ stands for the concentration of gas species j (O_2 , CO_2 and N_2) in the headspace (kg/m^3), V_{HS} is the volume of headspace (m^3), $\varphi_{j,L}$ is the mass flow (kg/s) of species j occurring through the lid film from the surrounding atmosphere to the headspace, $\varphi_{j,I}$ is the mass flow (kg/s) of species j (kg/s) at the interface between the food sample and headspace and $S_{j,F}$ is the net production rate (kg/s) of species j (O_2 , CO_2) due to microbial respiration/fermentation by biological reactions, if any, occurring within the food sample during conservation.

In the present work, the respiration of microorganisms, $S_{j,F}$ was modelled using the formalism of Micaëlis-Menten. The Micaëlis-Menten equation is a global approach that permits to predict the global O_2 consumption and CO_2 production by the microorganisms in the product.

$$S_{j,F} = \left(\frac{r_{O_2,max} \cdot p_{O_2,HS}}{K_m + p_{O_2,HS}} \right) \bar{N}_t \cdot m \quad (2)$$

Where $r_{O_2,max}$ is the maximum respiration rate ($kg\ s^{-1}\ CFU^{-1}$), K_m is the Michaelis-Menten constant (Pa), $p_{O_2,HS}$ (Pa) is the partial pressure of O_2 in headspace, \bar{N}_t is the microorganism concentration ($CFU\ g^{-1}$) and m is the mass of the food (g). It was assumed that the production of CO_2 was equal to the consumption of O_2 in the present study. $r_{O_2,max}$ and K_m were estimated from the work of (Thiele et al., 2006) who modelled the respiration of *Pseudomonas fluorescens* at 7°C from dedicated respiration experiments.

The logistic model proposed by Rosso (Rosso, 1995) was chosen as the primary model to describe microbial growth. In secondary model, the effects of temperature, pH and aw were modelled using the gamma concept (Zwietering et al., 1992) and that of CO_2 and O_2 as shown in the following. For CO_2 :

$$\gamma_{CO_2}(x, T) = 1 - \frac{C_{CO_2,F}(x, T)}{C_{CO_2,max}} \quad (3)$$

where $C_{CO_2,F}(x, T)$ is the concentration of dissolved CO_2 (kg/m^3) into the food sample at a given position x and time t and $C_{CO_2,max}$ is the maximal concentration of CO_2 (kg/m^3) withstanding by the microorganism (above this value, no growth occurs). $C_{CO_2,max}$ is calculated from the $\%CO_{2,max}$ (%) as follows:

$$C_{CO_2,max} = \%CO_{2,max} p_T M_{CO_2} k_{H,CO_2} \quad (4)$$

Where k_{H,CO_2} is the CO_2 solubility coefficient (according to Henry's law) in $mol\ Pa^{-1}\ m^{-3}$ and M_{CO_2} is the molar mass of CO_2 in $kg\ mol^{-1}$.

For O_2 , the γ_{O_2} parameter was calculated using the Monod model

$$\gamma_{O_2}(x, T) = \frac{C_{O_2,F}(x, T)}{C_{O_2,min} + C_{O_2,F}(x, T)} \quad (5)$$

where $C_{O_2,F}(x, T)$ is the concentration of dissolved O_2 (kg/m^3) into the food sample at a given position x and time t and $C_{O_2,min}$ (kg/m^3) is the minimal concentration required for microbial growth.

Numerical solving. The system of differential equations (see (Chaix et al., 2015a) for more details about discretization of equations) is solved using a dedicated algorithm "ode15s" developed in Matlab® computing software (The Mathworks Inc., Natick, Mass, USA) and adapted to stiff systems where each of unknown variables may exhibit radically different variation kinetics. This algorithm adjusted automatically the size of the time step used for numerical integration of the equations.

RESULTS & DISCUSSION

Model validation.

All the input parameters required in the model were either measured during the challenge tests (dimensions of the system, permeabilities to O₂ and CO₂, aw and pH of food, initial O₂ and CO₂ partial pressures in headspace, optimal growth rate for both microorganisms on the studied products) or taken from the literature (O₂ and CO₂ solubilities, diffusivities and activation energies for each coefficient, i.e. permeability, diffusivity and solubility) or taken in the SymPrevious database for microbial parameters (except CO₂ and O₂ effect – present work).

The model proposed necessitates no less than 50 input parameters for one simulation. But most of them are fixed such as energy of activations because they do not impact so much the simulation as revealed by a sensitivity analysis (Chaix et al., 2015a). Finally, 11 parameters are determinant in the simulation and must be carefully determined for each case such as O₂/CO₂ permeation, CO₂ solubility into the food, microbial parameters such as optimal growth rate and some parameters of secondary models such as $C_{CO_2,max}$. The temperature of storage heavily impacts the simulation and especially the microbial growth curve and must be known precisely.

Preliminary analysis of challenge test results has revealed that we cannot neglect the respiration of microorganisms (*Pseudomonas* respiration) and its effect on evolution of internal O₂ and CO₂ concentration in headspace. Even when O₂ was removed from the pack, there are still some residual amounts of O₂ (about 2%) due to desorption from the product and imperfect gas flushing. This initial O₂ concentration in each case rapidly decreased as illustrated in Fig. 1. CO₂ concentration (30, 50 or 60% initial concentration) also rapidly drops into headspace (Fig. 1). This decrease is the interplay of different mechanisms namely (1) dissolution into the food product, (2) loss by permeation through the packaging material and (3) production by microorganism through aerobic respiration. Therefore, respiration modelled by Mickaëlis-Menten equation was added in the model. Respiration parameters determined by (Thiele et al., 2006) for *Pseudomonas fluorescens* were used. Taking into account the respiration in the simulation, the model succeeded in predicting evolution of O₂, CO₂ and N₂ internal partial pressures during storage (4, 8 and 15°C), as shown Fig. 1 (solid lines). Preliminary simulations have highlighted that the value of CO₂ concentration initially dissolved in the food was highly impacting on the predicted CO₂ partial pressure and consequently on the predicted microbial growth. However, this value could not be measured during experiment and hypothesis on this value must be done. In the case of poultry, the initial CO₂ content dissolved in the meat was estimated to be approximately 1/3 of the headspace content.

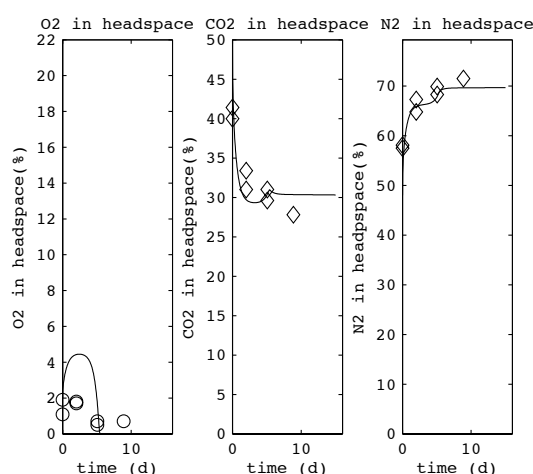


Figure 1: Example of experimental (symbols) and predicted (solid lines) evolution of O₂, CO₂ and N₂ concentration in headspace of poultry initially packed in 50% CO₂/50%N₂ at 8°C

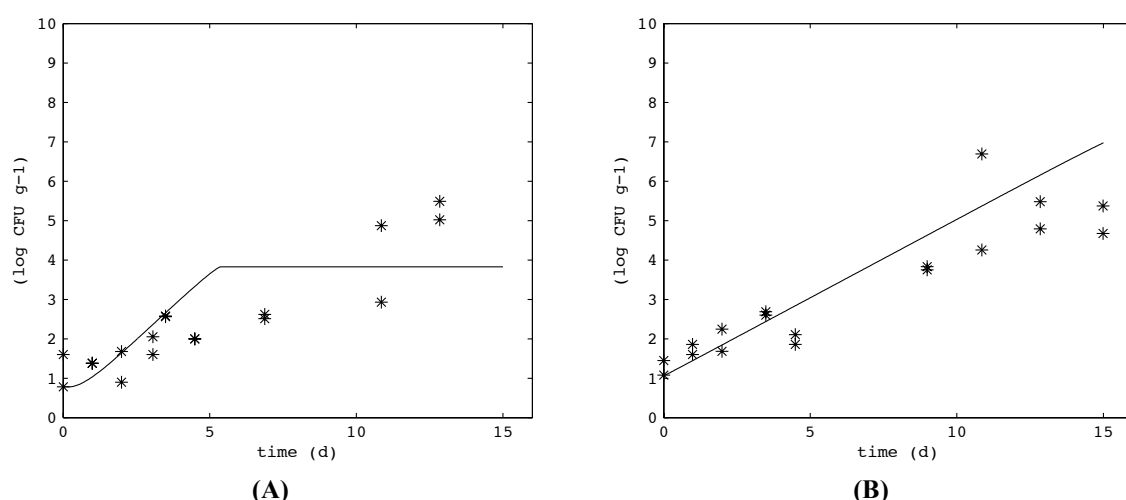


Figure 2: Example of experimental (symbols) and predicted (solid lines) microbial growth on food surface (poultry) initially packed in 50% CO₂/50%N₂ at 8°C: (A) *Pseudomonas fluorescens* and (B) *Listeria monocytogenes*

Predicted microbial growth was then compared with experimental ones (Fig. 2). The model predicted well the microbial behaviour without any adjustment of any parameters. We nevertheless noticed some slight deviations at the end of experiments (see *Pseudomonas* growth curve at 8°C, fig. 2 -A-). This was related to the aging of the product which exceeded its shelf life. Two clearly different behaviours were observed for *Pseudomonas* and *Listeria*. While *Pseudomonas* was clearly affected by CO₂ content (with a $C_{CO_2,max}$ equal to 40%), *Listeria* was not affected at all by CO₂ content ($C_{CO_2,max}$ equal to 304%). In addition, *Pseudomonas* was strongly affected by the O₂ content while *Listeria* was not. As respiration occurs in the pack, anoxia happened between 5 and 10 days (depending on the level of microorganisms) following by a sudden stop in growth rate and a plateau on the growth curve of *Pseudomonas*, clearly obvious on Fig. 2 -A-. This feature highlighted that respiration occurring in the system could trigger the anti-bacterial effect of CO₂ by creating anoxia.

The model once validated on poultry meat was also tested on processed cheese inoculated with *Pseudomonas fluorescens* and stored at 4 or 8°C in MAP containing either 60% CO₂/10%O₂/30% N₂ or 30% CO₂/70% N₂. The model succeeded in predicting the growth rate on cheese but with much more uncertainty (results not shown) that would be related to the $C_{CO_2,max}$ and $C_{O_2,min}$ measured for this bacteria.

Decision Support Tool. Once validated the aforementioned model could be used as a Decision Support Tool to help the user to properly design its system (internal atmosphere composition, ratio food volume to headspace volume, etc) and to rationally select its packaging material using a requirement driven approach (Fig. 3). Such approach starts from the needs of the product (i.e. maximal level of bacteria at shelf-life translated into optimal storage atmosphere) to the identification of packaging properties which permits to select the suitable material in a data base. The MAP modelling tool previously presented permitted to adequately transcript the needs of the produce (i.e. maintaining the protective atmosphere in a given range of tolerance) into packaging permeabilities. Demonstration of such approach is proposed in the following.

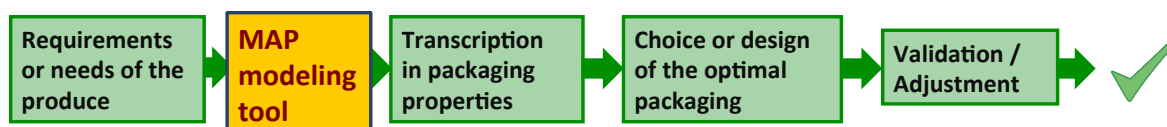


Figure 3: The basements of a Requirement Driven Approach: from the needs of the food product to the packaging

Supposing that you want to store some processed poultry meat between 4 and 6°C in MAP containing 50% CO₂, 2% of residual O₂ and 48% of N₂. The maximal load tolerated on your product at the end of the use-by-date (20 d) is 10⁷ CFU / g (hypothetical value) for an initial load of 10³ CFU / g (hypothetical value). The MAP modelling tool previously described permits to test some values of O₂ and CO₂ permeabilities and to identified some couples of suitable values for this application (Fig. 4). As shown on Fig. 4, the values of 4.28x10⁻¹⁷ for O₂ permeability and 1.71x10⁻¹⁶ mol/m/s/Pa seem to fit the shelf life criteria for the case study. The second step is to find the packaging materials displaying such values of permeabilities. This could be done by query our packaging database (more than 350 permeability values capitalised on the present date). A dedicated web application @Web was used to build the query and find the ranking of the most suitable packagings as shown in Fig. 5. In the case of the present case study, the query made on O₂ permeability value has given more than 8 materials in answer, two being ranked in first position (Fig. 5). Last step is to test this material for a final validation of the selection made by the Decisions Support Tool. This requirement driven approach is very efficient for helping food manufacturers in identifying rapidly materials for their MAP application, saving cost and time.

Initial: 10³ UFC/g

End of use-by-date: 10⁷ CFU/g,

Storage : (1/3) 4°C et 6°C (2/3)

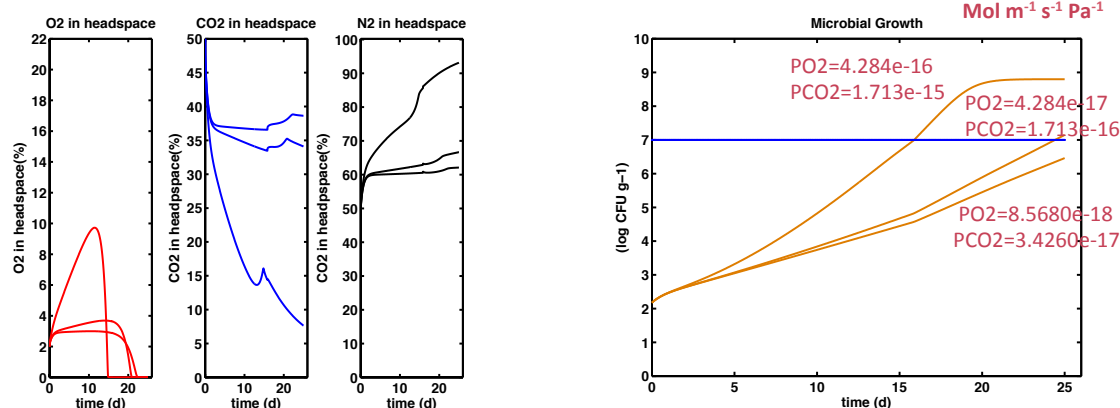


Figure 4: Case-study: identification of the couple of suitable O₂ and CO₂ permeability for the storage of poultry in order to obtain with an initial atmosphere of 50% CO₂ and 2% O₂ a quantity of *Pseudomonas* at the end of use-by-date lower than 10⁷ CFU / g, initial load 10³ CFU / g (hypothetical data)

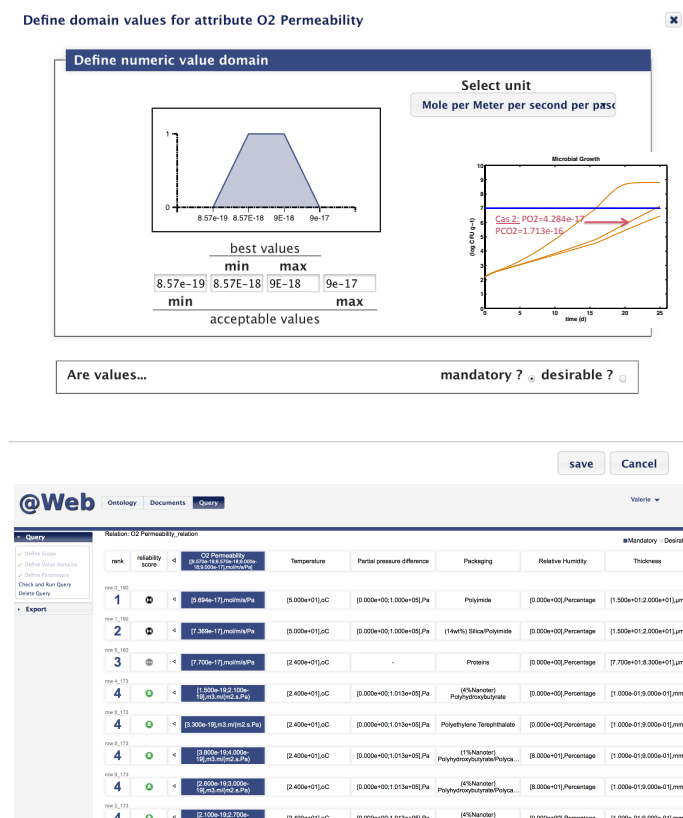


Figure 5: Building of a query for packaging database on @Web (above) and result of this query (below)

CONCLUSION

The mathematical model proposed here coupled mass transfer equations with predictive microbiology. It permits to efficiently predict the evolution of O_2 and CO_2 concentration in headspace and microbial growth on (or in) the product. It was validated on real products via challenge tests. The comparison of model prediction with experimental data has revealed that:

- respiration could not be neglected in the system and must be modelling to accurately predicted the dynamic of gas changes and microbial growth. The respiration could eliminate residual O_2 in headspace and therefore trigger the anti-bacterial effect of the initial chosen atmosphere;
- input parameters are numerous and some of them could not be precisely determined (as initial dissolved CO_2 content in food) and therefore have to be estimated;
- it was confirmed that *Pseudomonas* is more sensitive to CO_2 than *Listeria*. The effect of O_2 on *Pseudomonas* could not be neglected.

The MAP modelling tool developed here was proved to be efficient as a Decision Support Tool for helping the user in the design of his MAP system. An effort must be brought in the future on the consolidation of existing databases gathering data for simulation (e.g. microbial parameters) and for packaging data.

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